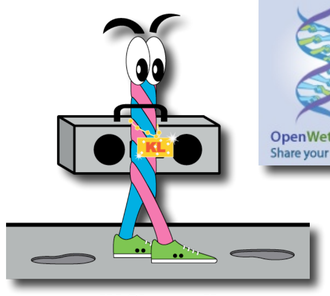

Speed effects on gliding motility assays due to surface passivation and water isotopes.

Andy Maloney, Larry Herskowitz, Michael Wilson, Steve Koch.
University of New Mexico

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Surface passivation effects

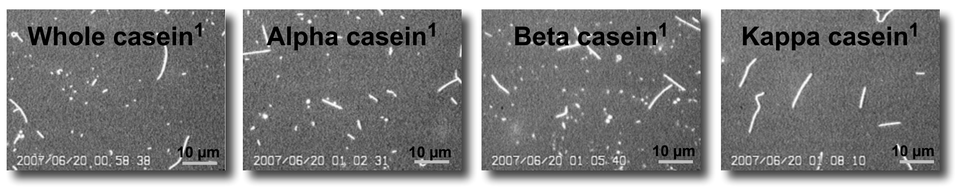
Motivation

For kinesin to function properly in a gliding motility assay, the substrate to which kinesin adheres must first be passivated[1]. Functionalization of glass slides and slips is typically performed with bovine milk proteins called caseins, however, BSA can also be used as a passivator[7]. Bovine casein is a globular protein that can be broken up into four major constituents: α_{s_1}, α_{s_2}, β, and κ. Each casein constituent affects how kinesin adheres to the glass (or possibly how kinesin adheres to casein) and they ultimately affect the speed at which microtubules are observed to glide. Building on the work of Verma et. al.[1], we have found that each constituent of bovine casein produces different outcomes in gliding assays.

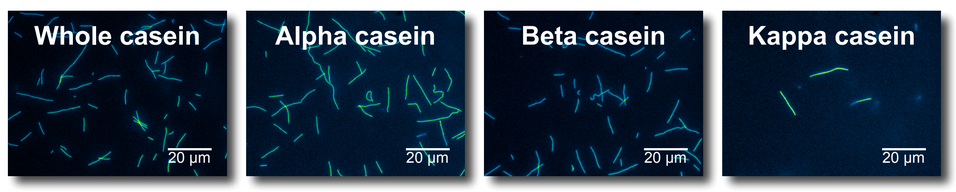
Experiment

All chemicals were purchased from Sigma unless stated otherwise. In order to determine the differences in how kinesin and microtubules interact dependent on casein constituents used as passivators, we first introduced caseins to our base buffer PEM [80mM PIPES (Sigma-80635), 1mM EGTA (Sigma-03778), 1mM $MgCl_2$ (Sigma-M1028), pH 6.89] at 0.5mg/mL. α casein, (a mixture of α_{s_1}:α_{s_2} ~ 7:2[2], Sigma-C6780), β casein (Sigma-C6905), and κ casein (Sigma-C0406) go into solution easily with constant stirring, however, whole casein is notoriously difficult to get into solution. There exists a great deal of legacy procedures in the community on how to get whole casein into solution, some of which include extended periods of centrifugation and filtration. Whole casein does not have a secondary structure and will not denature under heat[3]. Thus, to get whole casein into PEM, we heat the solution and stir in 0.5mg/mL whole casein until it is completely dissolved since mild heating cannot affect the casein micelle structure. Finally, all casein solutions are passed through a 0.2µm filter to remove any possible bacteria that may be in the solution.

Results

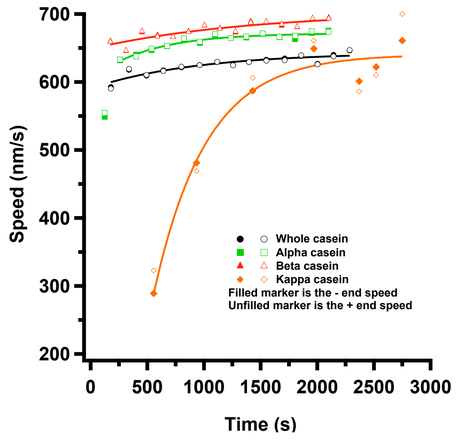


Very nice work done by Verma et. al.[1] (above) shows that microtubules are supported differently in the gliding motility assay dependent on the type of casein used as a passivator. Building on what they have done (below), we investigated the speed changes microtubules glide at depending on what type of passivation is used. Our preliminary work notes that kappa casein is by far the worst at supporting the gliding motility assay at the kinesin concentration we use, 30µg/mL. Images are false colored using ImageJ.



Data analysis

Microtubules are tracked using custom LabVIEW software. Data is taken from a total of 15 regions in each slide. Each ROI, and correspondingly each data point, is illuminated for approximately 2 minutes. The illumination source is a 100W Hg lamp that is attenuated by 94% and sent through a standard TRITC filter cube. Data is smoothed using a sliding Gaussian window to eliminate Brownian noise. We then determine the probability desinty function for instantaneous microtubule speed from a kernel density estimation using a Gaussian kernel of width 50nm/s.



The peak of the PDF is used as the speed for that ROI. An interesting feature of the data is that the microtubules speed up over time in all assays. This feature has led us to stabilize the temperature of our objective. Subsequent measurements have shown that stable speeds can be obtained with temperature stabilization. We are currently investigating if any differences occur with different passivations and temperature stabilization.

Water isotope effects

Motivation

Water plays an important role in biological systems and it is often overlooked[4]. It is a key component for the hydrolysis of ATP and has a crucial role in how proteins interact with one another due to the hydration of their surfaces. To the best of our knowledge, no work has been done on investigating how the kinesin-1 microtubule system changes when changes with water isotopes occur. Changing the water isotopes from hydrogen to deuterium or ^{16}O to ^{18}O may affect how kinesin interacts with microtubules. This change can be used as a novel experimental knob to probe features of the kinesin and microtubule system in new and different ways.

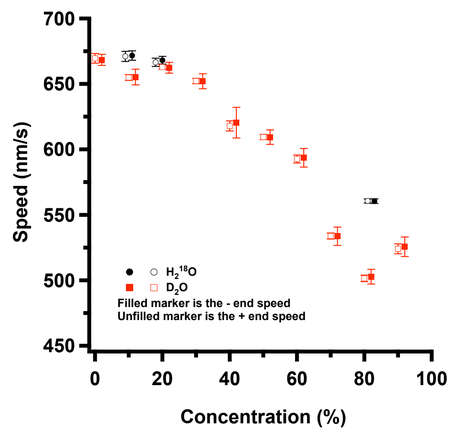
Experiment

All chemicals were purchased from Sigma unless noted otherwise. Slide preparation was performed as follows:

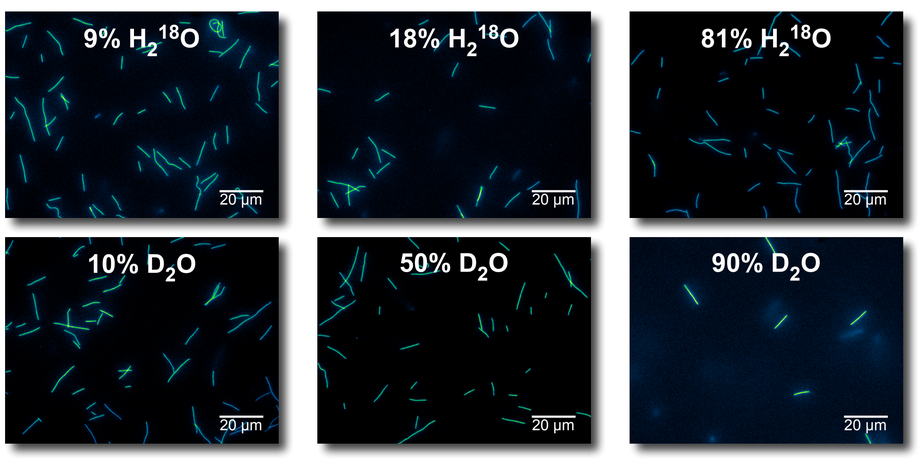
- 10 minute incubation of slide with 0.5mg/mL α casein in PEM.
- 5 minute incubation of slide with 0.25mg/mL α casein, 30µg/mL kinesin-1, and 1mM ATP in PEM.
- Introduction of Taxol stabilized microtubules with an enzymatic antifade system and 1mM ATP in PEM with the desired mix of water isotopes. After sealing, observations are conducted immediately.

When using D_2O, care must be taken in order to measure the correct "pH" denoted as the pD = pH + 0.41[5]. Also, all solutions that have Taxol in them are always prepared fresh and are discarded after 3 hours to prevent Taxol crystals from forming[6].

Results



- The tracking software is capable of tracking both the positive end (unfilled markers) and the minus end (filled markers) of the microtubule. Plus and minus ends are offset for clarity in the plot. We use the two tracked numbers as a check to ensure that the microtubules are tracked properly. See Larry Herskowitz's poster for a more detailed description of the tracking software.
- $H_2{}^{18}O$ (Sigma-329878, black markers, 97% ^{18}O) is quite expensive. We purchase this water 1 gram at a time. In order to prepare the PEM solution with $H_2{}^{18}O$ water, a 10x solution of PEM is prepared in regular H_2O. It is then diluted by a factor of 10 in $H_2{}^{18}O$. What is plotted above is the % concentration of $H_2{}^{18}O$ used in an assay and not the % concentration of ^{18}O in solution.
- While time prevented us from completing the experiment, You can clearly see the reduced speed trend by increasing $H_2{}^{18}O$ concentration in the assay.
- D_2O (Sigma-151882, red markers, 99.9% D) is not as expensive as $H_2{}^{18}O$ and so PEM is prepared in D_2O. Again, what is plotted is the % D_2O used in the assay and not the % deuterium in solution.
- A distinctive trend is observed as one reduces the amount of H_2O in the assay.
- Data points at 70% and 80% D_2O may be an experimental error and thus may not represent the true nature of the system.
- Error bars are the SEM of 8 ROIs where the slight increase in speed over the 8 regions is ignored (see data in the surface passivation section).



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